THE JOURNAL OF INVESTIGATIVE DERMATOLOGY 67:265–269, 1976

Vol. 67, No. 2
Printed in U.S.A.

SKIN FLORA MAPS: A TOOL IN THE STUDY OF CUTANEOUS ECOLOGY

DAVID J. BIBEL, PH.D., CPT, MSC, U.S. ARMY, AND DAVID J. LOVELL, SP6, U.S. ARMY

Department of Dermatology Research, Letterman Army Institute of Research, Presidio of San Francisco, California, U. S. A.

We sampled 162 and 175 skin sites, respectively, of a patient with severe atopic dermatitis and of a healthy subject, and constructed maps of the two individuals depicting the density and distribution of aerobic cutaneous flora over the entire body. All isolates of Micrococcaceae were biotyped. Neither the density nor the kinds of microorganisms were homogeneously arrayed. Instead, the separate types of flora were skewed in distribution, tended to segregate on large anatomical regions, and inhabited overlapping territories. No two sites were exactly alike in their carriage of microorganisms. Many of the apparently normal skin sites of the eczema patient carried high numbers of *Staphylococcus aureus*, which often was the dominant organism. Skin flora maps are seen as a potentially useful tool, especially in studying the dynamics of cutaneous microbial populations.

To our knowledge no map depicting an individual's cutaneous flora has been published. Detailed maps can help determine which sites, if any, are representative for the entire body, what is the degree and consistency of variation among symmetrical sites, and whether the distribution pattern can be correlated with major anatomical regions. Maps of patients, especially those with dermatoses, may show characteristic effects upon the normal flora. Furthermore, such maps can be especially useful in studying the dynamics of the cutaneous microbial population under various environmental stresses, in much the same manner as with other defined ecosystems, and in determining whether people can be typed ecologically according to their resident flora.

Knowledge of the extent and nature of microbial variation is pertinent to the understanding of cutaneous ecology and the role of skin flora in disease. Whereas the degree of variation among individuals was not fully appreciated until the introduction of Baird-Parker's [1,2] scheme for biotyping staphylococci and micrococci, insufficient attention has been given to the individual ecosystem. Yet most workers in this field can attest that one's carriage of normal flora seems to be unique or at least to belong to numerous, but still undefined, ecologic groups. Now that basic information has been obtained regarding the flora found within human populations, emphasis can be directed towards studies of the individual.

Until recently, only large, nonquantitative surveys have been conducted to gather prevalence data on the inhabitation of Baird-Parker biotypes on few, selected anatomical sites [3–5]. Alternatively, the flora of several areas of the body have been enumerated in some studies at the expense of classification [6,7]. Because of technical difficulties, both differentiation, even partial, and quantitation have been achieved in only a limited number of investigations [8,9]; however, we have developed a procedure [10–12] which may greatly expand this type of work. The direct result of such studies is a more complete analysis of individual flora.

In 1965 and 1969 Marples [13,14] drew distorted human figures displaying the relative density and distribution of cutaneous flora as determined by various laboratories. Unfortunately, the number of studies and representative sites was small, sampling techniques differed, and microorganisms were incompletely classified. The illustrations were meant to show typical patterns and were not designed to be accurate maps of the "average" person. Nevertheless, they were visually effective and instructive.

This report presents, as examples, flora maps of 2 subjects, one with atopic dermatitis and the other healthy. Despite the extreme differences in the skin of the volunteers and in the composition of the flora, various common ecologic attributes were discerned.

MATERIALS AND METHODS

Volunteers. Two people freely consented to participate in this study. The first subject was a 38-year-old white female nurse who was suffering mutiple lesions of atopic dermatitis. Three weeks prior to sampling she began treatment with systemic corticosteroids, but therapy was terminated two weeks later after apparent success. When she arrived at the laboratory, her inflammation had returned and was very severe. She routinely used a nondeodorant soap and an axillary antiperspirant containing aluminum chlorohydrate. The second volunteer was an apparently healthy, white male laboratory technician, age 26. For one week prior to sampling he washed

Manuscript received January 14, 1976; accepted for publication March 25, 1976.
Reprint requests to: Dr. D. J. Bibel, Letterman Army Institute of Research, Box 126, Presidio of San Francisco, California 94129.

with a nondeodorant soap, but used an axillary deodorant spray contining benzalkonium chloride and zinc phenosulfonate. These subjects were chosen as examples, but not necessarily representatives, of ecosystems with healthy or diseased skin.

Mapping. An arbitrary grid was drawn upon the body of the volunteer using a pen (Skilcraft, Houston, Texas) whose water-soluble ink was neither toxic, antimicrobial, nor a source of contaminating microorganisms. In general, the bottom of the foot was divided into 3 segments; the calves were drawn into 9 parts with the tibia marking the apex of the 3 sets of triangles; patella and popliteal areas were delineated; the chest and abdomen were split along the sternum-umbilicus axis; the forearm was separated into 6 portions with longitudinal junctions running along the palmar-dorsum boundary; elbow, antecubital spaces, axillae, periumbilicus, and other areas of the skin were marked in a similar fashion (Figs. 1, 2). The fourth toe webs and fourth finger tips were also sampled, but genitalia were not included. No zone measured greater than 10 × 10 cm, and each area was assigned an identifying number.

Besides constructing a sketch showing the encoded network, photographs were taken of front, back, and side views. After highlighting the boundaries of the grid, photographs were placed in a projector and the image traced on paper. Although a fairly accurate map can be achieved, body outlines and facial features were altered to maintain the privacy of volunteers for this paper. Once the basic surface map was prepared, each area was marked according to the density of its specific flora.

Sampling. Neither subject bathed or showered the morning of sampling. Wherever possible, we attempted to sample the center of each grid. A sterile stainless-steel well, measuring 1.5 × 1.5 cm, was held tightly against the skin and filled with 1 ml of phosphate-buffered, pH 7.9, Triton X-100 [6]. The skin was then scrubbed back and forth for 25 cycles using a sterile, blunted, stainless-steel spatula. To ensure a standard pressure of friction, a 250-gm weight was attached to the handle of the scraper. The system corresponds to our linear friction machine used in previous studies [10–12]. After drawing up the turbid liquid, another 1 ml of buffer was added and scrubbing was repeated for 25 cycles. Again 1 ml of buffer was placed into the well as final rinse, and the pooled samples were diluted in 10-fold amounts for subsequent inoculation in triplicate upon plates of trypicase soy agar [10]. The system is sensitive to 4 colony-forming units/cm^2.

For the few sites of small size, such as toewebs, fingertips, and umbilicus, a moistened calcium alginate swab was used for sampling. After scrubbing, the specimen was dispersed in 3 ml of buffer, diluted, and plated.

Identification. Only the aerobic bacterial flora was studied. Technical problems prevented the isolation and identification of the anaerobic Propionibacteria and the Pityrosporum species. All isolates on the selected dilution plate were enumerated and identified using a replica-plating procedure [10]. Micrococcaceae were classified to the biotype level. Diphtheroids were separated according to size of colonies—a clear differentiation. Gram-negative organisms were speciated by standard methods [15].

Time and labor requirements. The tasks of preparing media and identifying isolates from each subject were completed in two weeks but necessitated three workers laboring full time. Two days alone were required to obtain and plate specimens when the body above or below the waist was sampled per day. The production and analysis of maps brought the study to three weeks.

RESULTS AND DISCUSSION

In this investigation 162 and 175 samples were obtained, respectively, from the atopic and normal volunteers. The disparity in numbers of sites was due to differences in their total surface area. Viewing the assorted maps (Figs. 1, 2*), one may immediately observe the heterogeneous arrangement of flora and may also readily notice the lack of consistency in location and density of flora on anatomically similar and symmetrical sites. This is especially apparent in the second volunteer. All but 16 sites were unique according to the proportional groups shown for each organism. Of these exceptions, 13 were composed of 4 groups of adjacent sites: 3 sets of 2 and a set of 3 juxtaposed areas. No two anatomically symmetrical sites had the same pattern of flora. Because of the fewer number of biotypes carried by the eczema patient, many more adjacent and even some symmetrical sites were of the same graphic pattern, but in both cases, when exact numbers were considered, no 2 sites were alike. Thus, with simplification of flora, as well as with less refined classification, one is more apt to find symmetrical distribution.

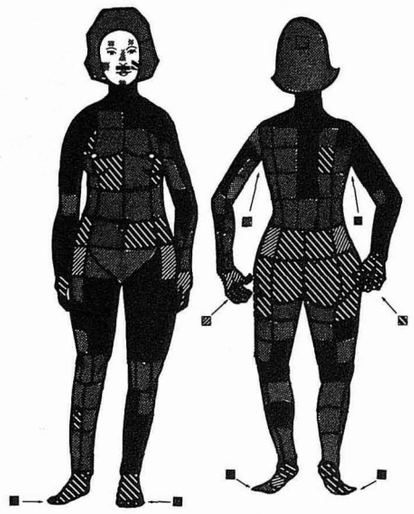
Since symmetry was not consistently present in either subject, we wondered whether there was a general left- or right-handed distribution. There was a higher proportion of flora on the left side of the healthy subject in 8 of 11 tests or maps, and the number of sites was significantly different by the χ^2 test ($p < 0.02$). No such trend was found with the eczema patient. Many more individuals will have to be mapped several times to determine whether overall or regional skewed patterns are common and constant.

The distribution of flora among diverse anatomical areas is another matter, and large differences can be found in both microbial presence and density. The distribution pattern of each kind of microorganism forms its territorial domain or range. These territories overlap. They are not random but are a manifestation of the organism's interaction with other flora, the host substrate, and microclimate, for, like higher life forms, bacteria

* Because of space limitations, maps displaying the distribution of some kinds of flora have been deleted. They may be obtained from the senior author.

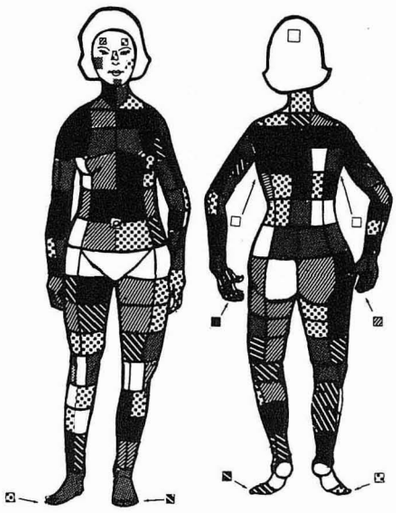
FIG. 1. Skin flora maps of a patient with severe atopic dermatitis. This is an example of an ecosystem with diseased skin, but may not be representative. The distribution of the atopic dermatitis was similar to that of total aerobic flora and of *Staphylococcus aureus*. The subject also carried *S. epidermidis* biotype 4 and *S. saprophyticus* biotype 1. *S. saprophyticus* biotype 2, *S. epidermidis* biotype 3, *Enterobacter aerogenes*, *Acinetobacter calcoaceticus*, and Pseudomonas species were detected also. Maps of these organisms are available from the senior author.

S. AUREUS



TOTAL AEROBIC FLORA
CFU / CM^2

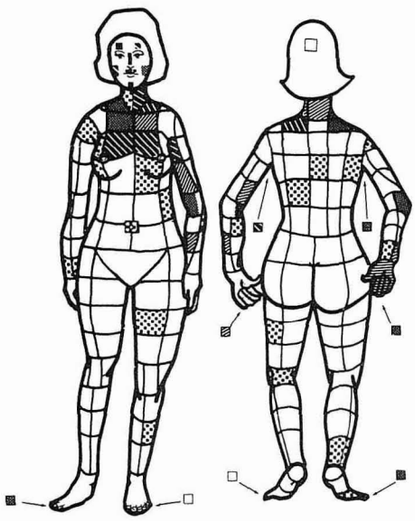
- 10^2
- 10
- 10^4
- $> 10^5$



PROPORTION OF FLORA

- NONE DETECTED
- 0.1 - 9 %
- 10 - 24 %
- 25 - 49 %
- 50 - 79 %
- ≥ 80 %

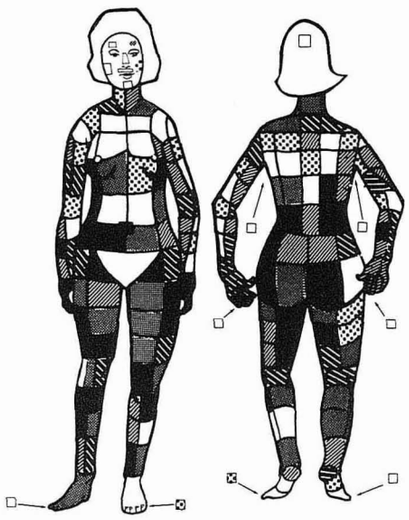
S. EPIDERMIDIS, BIOTYPE 1



PROPORTION OF FLORA

- NONE DETECTED
- 0.1 - 9 %
- 10 - 24 %
- 25 - 49 %
- 50 - 79 %
- ≥ 80 %

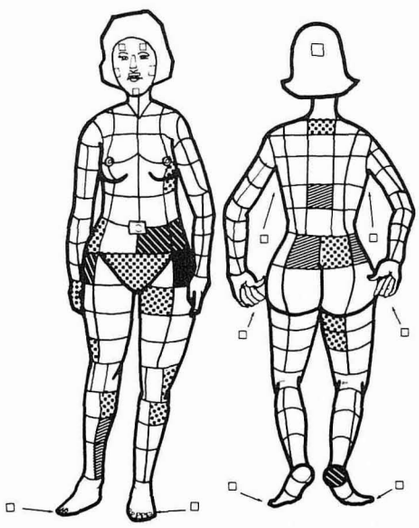
S. SAPROPHYTICUS, BIOTYPE 3



PROPORTION OF FLORA

- NONE DETECTED
- 0.1 - 9 %
- 10 - 24 %
- 25 - 49 %
- 50 - 79 %
- ≥ 80 %

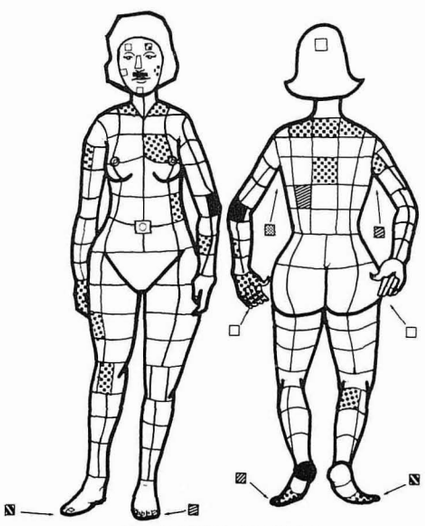
M. LUTEUS



PROPORTION OF FLORA

- NONE DETECTED
- 0.1 - 9 %
- 10 - 24 %
- 25 - 49 %
- 50 - 79 %
- ≥ 80 %

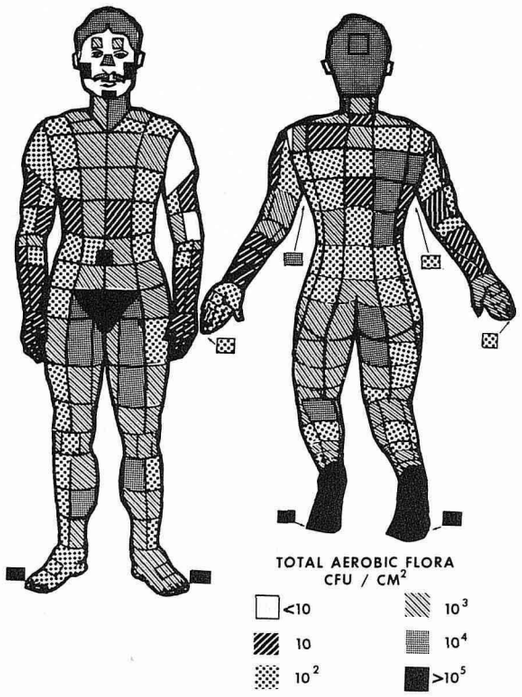
DIPHTHEROIDS



PROPORTION OF FLORA

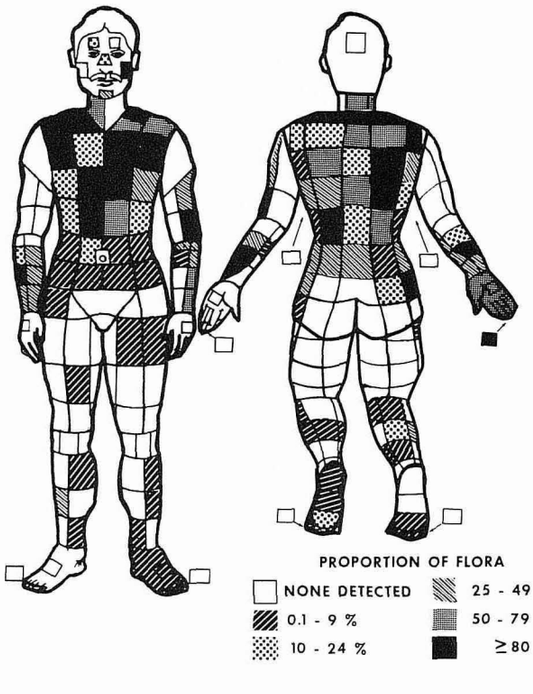
- NONE DETECTED
- 0.1 - 9 %
- 10 - 24 %
- 25 - 49 %
- 50 - 79 %
- ≥ 80 %

S. EPIDERMIDIS, BIOTYPE 3



TOTAL AEROBIC FLORA
CFU / CM^2

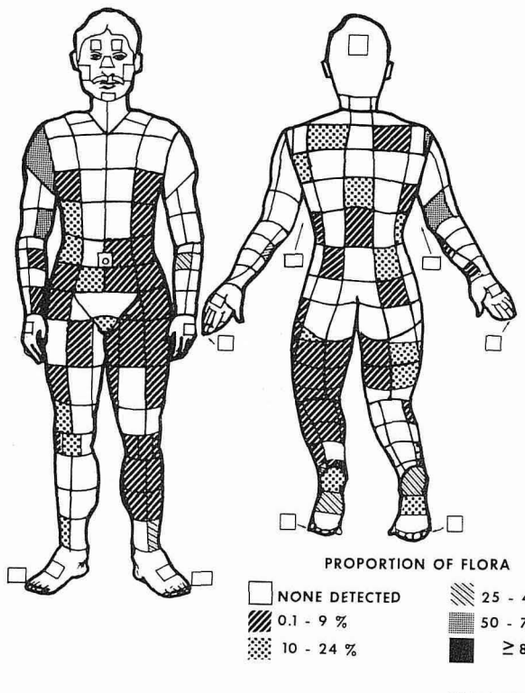
- <10
- 10
- 10^2
- 10^3
- 10^4
- $>10^5$



PROPORTION OF FLORA

- NONE DETECTED
- 0.1 - 9 %
- 10 - 24 %
- 25 - 49 %
- 50 - 79 %
- ≥ 80 %

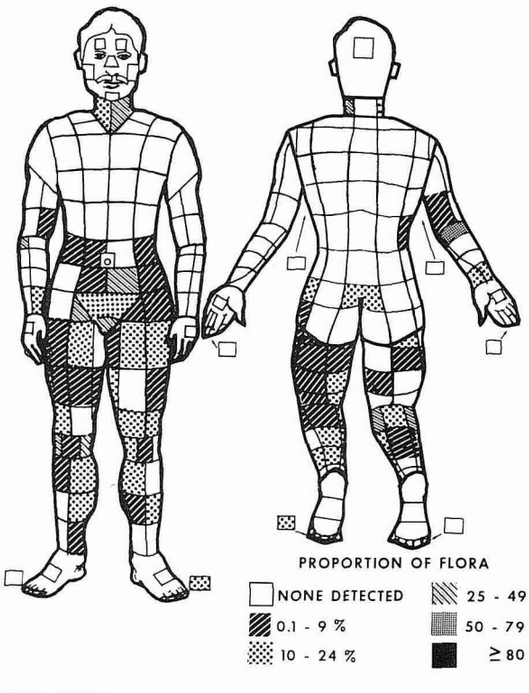
S. SAPROPHYTICUS, BIOTYPE 2



PROPORTION OF FLORA

- NONE DETECTED
- 0.1 - 9 %
- 10 - 24 %
- 25 - 49 %
- 50 - 79 %
- ≥ 80 %

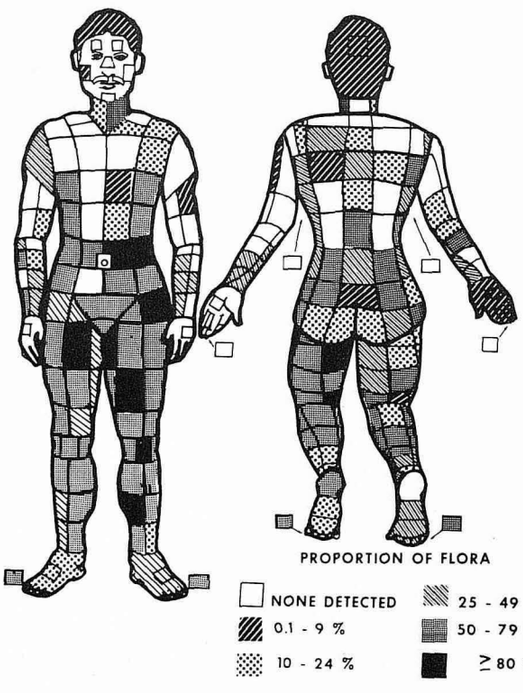
LARGE COLONY DIPHTHEROIDS
(MAINLY C. XEROSIS)



PROPORTION OF FLORA

- NONE DETECTED
- 0.1 - 9 %
- 10 - 24 %
- 25 - 49 %
- 50 - 79 %
- ≥ 80 %

SMALL COLONY DIPHTHEROIDS



PROPORTION OF FLORA

- NONE DETECTED
- 0.1 - 9 %
- 10 - 24 %
- 25 - 49 %
- 50 - 79 %
- ≥ 80 %

must compete and fill a niche for survival [16]. With respect to total flora, both subjects possessed the usual [7,14] high levels on toe webs, umbilicus, axilla, inner thighs, perineum. soles, neck, and face.

The eczema patient was chosen as an example of an altered ecosystem, and although it is not appropriate to compare the distribution of flora directly with that of the healthy subject, some conclusions can be drawn from the maps. In general, the greatest numbers of bacteria were isolated from the areas of inflammation, and *Staphylococcus aureus* was by far the dominant microorganism. Interestingly, *S. aureus* was detected in high numbers on areas of skin which were apparently normal. Only 25 sites of the 162 examined were free of the organism. Taking 10^5 colony-forming units/cm^2 as an average, a presence upon 85% of the skin surface, and a calculated surface area of about 17,600 cm^2 [17], one would find that the individual carried on her skin 1.5×10^9 cfu of *S. aureus*.

Skin flora maps have a great potential in studying cutaneous ecology, and a graphic display often is superior in handling information. The time and labor involved requires careful planning of experiments and surveys. However, whole-body maps may not be necessary for some purposes, and restricted zones, such as the back or legs, may be experimentally sufficient. Despite these demands, flora maps may become a useful and worthy tool, especially in studying dynamics. Thus far, it appears than skinborne microorganisms are not homogeneously arrayed either by density or by kind, may be skewed in distribution, tend to segregate on large anatomical regions, and have overlapping territories whose areas of concentration tend to peak. Body regions with the most diversified flora seem to be the back, legs, soles, and forearms. Areas with the most restricted flora apparently include the scalp, thorax, and umbilicus. It is clearly indicated that each small patch of skin is ecologically unique and that people probably are similarly special in their skin carriage of microorganisms. Individuals some day may be organized into ecologic biotypes according to their normal flora. With future studies of this order a more precise definition of the microbial ecology of the skin eventually can be attained.

We are grateful for the excellent technical assistance of SP7 Roko J. Smiljanic and the clinical support of LTC Joseph H. Greenberg, M.C.

REFERENCES

1. Baird-Parker AC: A classification of micrococci and staphylococci based on physiological and biochemical tests. J Gen Microbiol 30:409–427, 1963
2. Baird-Parker AC: The classification of staphylococci and micrococci from world-wide sources. J Gen Microbiol 38:363–387, 1965
3. Noble WC: Skin carriage of the Micrococcaceae. J Clin Pathol 22:249–253, 1969
4. Deacon AG, Smith JMB: Staphylococci and micrococci on the skin of infants. Proc Univ Otago Med School 51:6–7, 1973
5. Pasridia A, Bhujwala RA, Shriniwas: Applicability of Baird-Parker classification to strains of Micrococcaceae isolated from pyodermic patients. Indian J Med Res 61:976–979, 1973
6. Williamson P: Quantitative estimation of cutaneous bacteria, Skin Bacteria and Their Role in Infection. Edited by HI Maibach, G Hildick-Smith. New York, McGraw-Hill, 1965, pp 1–12
7. Ulrich JA: Dynamics of bacterial skin populations, Skin Bacteria and Their Role in Infection. Edited by HI Maibach, G Hildick-Smith. New York, McGraw-Hill, 1965, pp 219–234
8. Marples RR: The effect of hydration on the bacterial flora of the skin, Skin Bacteria and Their Role in Infection. Edited by HI Maibach, G Hildick-Smith. New York, McGraw-Hill, 1965, pp 33–41
9. Noble WC, Somerville DA: Microbiology of Human Skin. London, Saunders, 1974, pp 329–331
10. Bibel DJ, Smiljanic RJ, LeBrun JR: A replica plating method for the identification of Micrococcaceae. Can J Microbiol 21:1676–1680, 1975
11. Bibel DJ, LeBrun JR: Effect of experimental dermatophyte infection on cutaneous flora. J Invest Dermatol 64:119–123, 1975
12. Bibel DJ, LeBrun JR: Changes in cutaneous flora after wet occlusion. Can J Microbiol 21:496–500, 1975
13. Marples MJ: The Ecology of Human Skin. Springfield, Ill, Thomas, 1965
14. Marples MJ: Life on the human skin. Sci Am 220:108–115, 1969
15. Branson D: Methods in Clinical Bacteriology. Springfield, Ill, Thomas, 1972
16. Alexander M: Microbial Ecology. New York, Wiley, 1971
17. Documenta Geigy. Scientific Tables. Sixth edition. Edited by K Diem. Ardsley, NY, Geigy Pharmaceuticals, 1962.

FIG. 2. Skin flora maps of a healthy volunteer who provides an example, not necessarily representative, of an ecosystem with normal skin. Because of space limitations, maps of *S. saprophyticus* biotypes 1 and 3, *S. epidermidis* biotypes 1, 2, and 4, and various other bacteria have been deleted. Maps of these organisms may be obtained from the senior author.